Influence of gender and social environment in an animal model of affective disorders

Westenbroek, Christel

3

Gender-specific effects of social housing on chronic stress-induced limbic Fos expression

Christel Westenbroek, Johan A. den Boer and Gert J. Ter Horst

Department of Psychiatry, section Biological Psychiatry, Graduate school of Behavioral Cognitive Neurosciences, University of Groningen, PO box 30.001, Groningen, the Netherlands

Neuroscience 121: 189-199, 2003

Stress plays an important role in the development of affective disorders. Women show a higher prevalence for these disorders than men. The course of a depressive episode is thought to be positively influenced by social support. We have used a chronic mild stress model in which rats received foot-shocks daily for 3 weeks. Since rats are social animals we hypothesised that social housing, as a possible model for human social support, might reduce the adverse effects of chronic stress. Brain activity after chronic stress was measured in several limbic brain areas with the neuronal activation marker c-Fos. High behavioural activity due to housing rats under reversed light-dark conditions could be responsible for the observed high within group variability in some limbic regions. Fos-ir in the paraventricular nucleus of the hypothalamus was increased in all stress-exposed groups, except for the socially housed females who showed increased Fos-ir in control condition. Individually housed males and socially housed females, showed increased Fos-ir in the dorsal raphe. Amygdala nuclei were differentially affected by stress, gender and housing conditions. Also the mesolimbic dopaminergic system showed gender specific responses to stress and housing conditions. These results indicate that social support can enhance stress coping in female rats, whereas in male rats, group housing appears to increase the adverse effects of chronic stress, although the neurobiological mechanism is not simply a reduction or enhancement of stress induced brain activation.

Abstract

Introduction

Chronic stress and stressful life events are often linked with the onset of major depression, indicating the importance of stress in the development of mood disorders.[57] Stressful life events are associated with financial and employment problems in men and with network or interpersonal problems in women.[72] Also, the presence of social support has been positively linked to good mental and physical health. The level of perceived social support has been positively associated with the outcome of a depressive episode.[22,35] Even though clear gender differences have not been found, it has been suggested that social support, provided by women, but not men, reduce the stress-induced elevation of blood pressure and plasma cortisol levels.[30,41] Formal clinical support systems, like psychotherapy, have been shown to be effective in ameliorating depressive symptoms, either by itself or in combination with antidepressant treatment.[16,34] Psychotherapy also appears to normalise brain activity associated with symptom improvement,[4] providing indications for a neurobiological basis for social support/psychotherapy.

The life-time prevalence of affective disorders is higher in women.[40] Gender differences in responses to antidepressant treatment[43,47] and differences in brain activation to affective stimuli have been reported.[7,29] Apparently gender differences exist in the way the brain processes emotional stimuli. Female rats have higher stress sensitivity than males and also the estrus cycle affects stress responsivity.[25,77] In a number of studies opposite behavioural effects of stress have been reported in male and females. While males perform worse on memory tasks, female learning has been shown to improve after stress.[3,36] Stress has also been found to have a gender specific effect on the hippocampus, a brain area highly vulnerable to stress.[27,50]

Chronic stress exposure has been proposed as a valid animal model for affective disorders. Chronically stressed rats show a reduced anticipation to rewarding stimuli,[49] equivalent to anhedonia, which can be reversed with antidepressant treatment.[79] In addition these rats also demonstrate various sleep disturbances, another characteristic of depression.[11] Since rats are social animals, social housing of rats during chronic stress could provide a model to study the neurobiological effects of social support. Group housing is able to counteract the effects of a social defeat in male rats.[62,76] Also gender differences are found in the effects of housing conditions. While social instability affects females more than males,[32] crowding is stressful for males but it actually calms females.[5]

In the current study we investigated whether social housing and isolation differentially affect limbic brain activity of male and female rats following exposure to chronic footshock stress during 21 days, using c-Fos as a marker of neuronal activity.[64] Stress has been shown to induce Fos in many brain areas[44] and treatment

with antidepressants is able to modulate the Fos response to stress.[1,53] Previously we have shown that housing conditions differentially affect behaviour and Fos-expression in the raphe nucleus of male and female rats.[78]

Experimental procedures

Male (n=24) and female (n=24) Wistar rats where either individually (males: n=10, females n=10) or socially (males: n=14, females n=14) housed in unisex groups of 4 rats. Of the individually housed rats, 5 rats were subjected to chronic stress and 5 rats to a control treatment. From each social group, two rats underwent stress exposure and two served as controls (n=7 per group). To have an equal number of 4 rats in each cage, in two cages of both genders an extra rat was added.

At the start of the experiment rats were of the same age with males weighing 298 ± 3 g. and females weighing 214 ± 1 g. The light-dark cycle was reversed (lights on 19.00-7.00 hr) and water and food was provided ad lib. All experimental procedures were approved by the Animals Ethics Committee of the University of Groningen (FDC: 2509). Efforts were made to minimise the number of animals used and their suffering.

The estrus cycle of the females was monitored by stroking them gently on the back, which during estrus produced lordosis behaviour, accompanied by weight loss on the day of estrus.

Rats were subjected to a daily footshock protocol for 3 weeks. Rats in the stress group were transferred to a footshock box and received 5 inescapable footshocks during a session (0.8 mA in intensity and 8 sec in duration). A light signal (10 sec) preceded each footshock adding a 'psychological' component to the noxious event. This way we could also avoid the noxious stimulus on the last day of the procedure when only the light signal was given. The shock interval varied in each session, as well as the time of the day of the exposure and duration of the session, which varied from 30 to 120 minutes. Control rats were placed in similar, non-electrified, cages. The rats were regularly weighed allowing calculations of weight gain changes from day one of the procedure.

The rats were sacrificed on day 22 using sodium pentobarbital anaesthesia (1 ml, 6%). The rats were transcardial perfused with 50 ml heparinised saline and 300 ml of a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer (pH 7.4), 2 hours after the start of the last exposure to the control or stress box. On the last day, the stress-exposed animals were subjected to the light stimulus only so Fos activation changes would reflect the 'psychological' aspect of stress exposure and not of a foot shock related pain response, that can activate the same or related circuitry.[69] The brains were removed and postfixed in the same fixative overnight at 4°C. Adrenal and thymus weights, corrected for body weight, were calculated and used as indication of the amount of stress perceived.

Following an overnight cryoprotection in a 30% sucrose solution, serial 40 μm coronal sections were made with a cryostat microtome and collected in 0.02 M potassium phosphate saline buffer (KPBS). Fos immunostaining was performed on free-floating sections. Sections were rinsed with 0.3% H_2O_2 for 10 minutes to reduce endogenous peroxidase activity, thoroughly washed with KPBS and incubated with the rabbit anti-Fos antibody (1:10,000, Oncogene Research Products, San Diego, CA) diluted in 0.02 M KPBS with 0.25% Triton X-100 and 2% Normal Goat Serum for 72 hours at 4°C.

After thorough washing, the sections were subsequently incubated for 2 hrs with biotinylated Goat-anti-Rabbit IgG (1:1000 (Vector Laboratories, Burlingame, CA) in 0.02 M KPBS) and avidin-biotin-peroxidase complex (Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA). After thorough washing, the peroxidase reaction was developed with a DAB-nickel solution and 0.3% H_2O_2. Sections were washed for 15 minutes in buffer and mounted with a gelatine solution and air dried, dehydrated in graded alcohol and xylol solutions and then coverslipped with DePeX mounting medium (BDH). To reduce staining artefacts or intensity differences the sections from all groups were processed simultaneously.

Fos positive cells in the prefrontal cortex, bregma +3.20 to +2.15 (infralimbic; IL, prelimbic; PL, anterior cingulate; AC,), paraventricular nucleus of the hypothalamus, bregma –1.08 to-1.78 (PVN), amygdala, bregma –2.00 to -2.85 (central; CeA, medial; MeA, lateral; LaA and basolateral; BLA part), accumbens +2.15 to 0.45 (core; NacC and shell; NAcS region), ventral tegmental area, bregma –5.25 to –6.06 (VTA), the dorsal raphe nucleus, bregma –7.10 to -9.25 (DRN), and the dentate gyrus of the hippocampus, bregma –2.45 to –4.20 (DG),[67] were blindly quantified using a computerised imaging analysis system. Dopaminergic neurons in the VTA were localised according to the description in The Rat Nervous System [23]. The selected areas were digitised by using a Sony charge-coupled device digital camera mounted on a LEICA Leitz DMRB microscope (Leica, Wetzlar, Germany) at 100x magnification. Regions of interest were outlined with a light pen, measured and the Fos positive nuclei were counted using a computer-based image analysis system LEICA (LEICA Imaging System Ltd., Cambridge, England). The resulted data was reported as number of positive cells/0.1mm^2. No left-right asymmetry of Fos immunoreactivity (ir) was found and therefore the mean ± standard error (SEM) for both sides were calculated.

Statistical analyses were done with SPSS (version 10.0), and $p \leq 0.05$ was considered significant. Weight gain for each gender was analysed with a repeated measures ANOVA with days as within subject factors and treatment (control or stress) and housing (individual or social) as between subject variables. Fos data were analysed with an univariate ANOVA with gender, housing and treatment as between subject factors. Sphericity assumed modelling, with Greenhouse-Geisser and Huynh-Feldt adjustments, was applied.[60]

Results

Weights

Weight gain was significantly affected by chronic stress in male rats ($F_{1,20} = 39.37$, $p \leq 0.001$), reducing the growth rate in both individually and socially housed males (resp. $F_{1,20} = 27.63$, $p \leq 0.001$ and $F_{1,20} = 12.17$, $p = 0.002$). However no significant housing effect was observed (Fig. 1A). There was a significant day effect ($p \leq 0.001$) with weight steadily increasing over the days and an interaction effect between day and treatment ($p \leq 0.001$) (Greenhouse-Geisser correction). In contrast, in females chronic stress had no significant effects on the growth rate but here an effect of

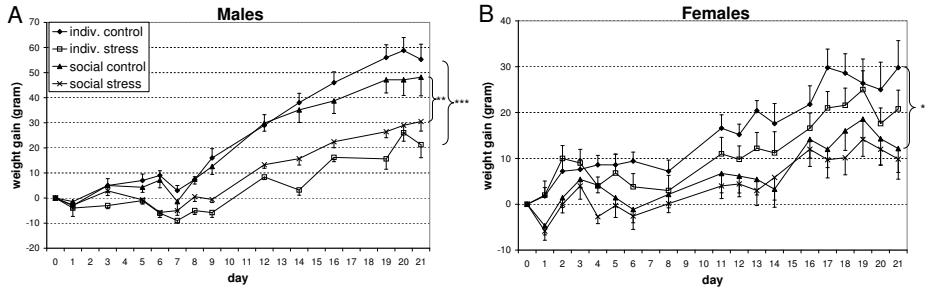


Figure 1. Weight gain in grams ± SEM. (A) Stress caused a significant reduction in weight gain of male rats (***, p≤0.001; **, p≤0.01). (B) Social housing reduced weight gain in control females (*, p≤0.05).

housing was observed ($F_{1,20}$ = 8.07, p = 0.010), socially housed control rats showing a reduced growth rate ($F_{1,20}$ = 5.11, p = 0.035) compared to individually housed control females (Fig. 1B). In females there was a significant effect of day (p≤0.001) and interaction effect between day and housing (p=0.034) (Greenhouse-Geisser correction).

Adrenal and thymus weights (Table 1)

Chronic stress had a significant effect on adrenal weight ($F_{1,32}$ = 10.26, p = 0.003), socially housed but not isolated male rats, showing adrenal hypertrophy after chronic stress exposure ($F_{1,16}$ = 6.85, p = 0.019). In females, stress induced a significant increase in adrenal weight in individually housed rats ($F_{1,16}$ = 4.60, p = 0.048) whereas in the socially housed females the adrenal weight was not increased. Besides stress effects, we found a gender difference in the relative adrenal weight. The female adrenal was significantly larger than the male adrenal ($F_{1,32}$ = 116.43, p≤0.001). Housing conditions alone had no significant effect on adrenal weight. Thymus weights were neither affected by stress nor by housing conditions.

c-Fos expression (Table 2)

Fos expression was analysed in the following areas, PVN, prefrontal cortex, dentate gyrus, amygdala, nucleus accumbens, VTA and dorsal raphe, since these areas have been associated with the stress response and abnormalities these regions have been implicated in affective disorders.

PVN: The PVN controls HPA-axis activity and the autonomic nervous system and showed a treatment ($F_{1,31}$ = 13.31, p = 0.001) and gender ($F_{1,31}$ = 11.89, p = 0.002) effect (Fig. 2B, 3C). **Male/females:** Pair wise comparisons showed that stress increased the Fos-ir in the PVN of indiv. and socially housed males (resp. $F_{1,12}$= 16.57, p = 0.002; and $F_{1,16}$ = 4.61, p = 0.047) and in indiv. housed females ($F_{1,12}$= 15.35, p = 0.002), but not in the socially housed females. ($F_{1,16}$=3,24, p=0,09).

Table 1. Relative adrenal weight (mg. adrenal/100 gr rat)

	indiv. controls	indiv. stressed	social controls	social stressed
Males	14.7(0.87)$^{\#}$	16.5(1.39)$^{\#}$	14.9(0.63)$^{\#}$	18.1(0.86)$^{*\#}$
Females	25.4(0.79)	32.1(2.89)*	26.2(1.30)	28.8(2.68)

Significant stress effects: *, p≤0.05, and significant gender differences ($^{\#}$, p≤0.05)

Gender differences: A gender difference in PVN Fos-ir was only observed in the individually housed rats (controls; $F_{1,12}$ = 11.04, p = 0.006; stressed: $F_{1,12}$= 12.07, p = 0.005).

Prefrontal cortex (PFC): The PFC is involved in many higher cognitive functions like motivation, working memory and attention and in the present study the Fos expression showed a treatment ($F_{3,30}$ = 4.96, p = 0.006) and gender ($F_{3,30}$ = 3.71. p = 0.022) effect. A gender, housing and treatment interaction was found for the infralimbic cortex (IL) ($F_{1,32}$= 5.23, p= 0.029). **Males:** Stress significantly increased Fos-ir of indiv. housed males in the different subregions of the PFC (IF: $F_{1,12}$= 14.50, p = 0.002; PL: $F_{1,12}$= 10.23, p = 0.008; AC: $F_{1,12}$= 6.62, p = 0.024). This was not observed in socially housed males, which we attribute to high levels of Fos-ir in socially housed controls, who showed a significantly higher basal Fos level in the IL ($F_{1,16}$ = 20.63, p ≤ 0.00), PL ($F_{1,16}$ = 22.18, p ≤ 0.00)(Fig. 3B) and AC ($F_{1,16}$ = 4,69, p = 0.046) than the isolated control males. **Females:** Fos-ir of socially housed rats was increased after stress in the IL ($F_{1,16}$ = 5.08, p = 0.039) and AC ($F_{1,16}$ = 6.19, p = 0.024). No housing effects were found. **Gender differences:** Isolated stressed males displayed a higher Fos expression in the IL ($F_{1,12}$= 4,85, p = 0.048) and AC ($F_{1,12}$= 5,63, p = 0.035) than the females and socially housed control males also showed more Fos positive cells than their counterpart females in the IL ($F_{1,16}$ = 19.12. p ≤ 0.00), PL ($F_{1,16}$ = 12,4, p = 0.003) and AC ($F_{1,16}$ = 4,70, p =0.046).

Dentate gyrus (DG): The DG is part of the hippocampus involved in learning and memory and sensitive to stress. Gender and housing had a significant effect on Fos-ir in the DG (resp. $F_{1,31}$ = 28.70 p = 0.000; $F_{1,31}$ = 14.69, p = 0.001). There was an interaction between housing and gender ($F_{1,31}$ = 9.61, p = 0.004) and a treatment effect in individually housed rats ($F_{1,12}$= 6.96. p = 0.022). **Males:** Chronic stress had no effect on DG Fos-ir in male rats, but the socially housed males showed significantly more Fos-ir than males living in isolation (controls: $F_{1,16}$ = 11.21, p = 0.004, and stressed: $F_{1,16}$ = 4,92, p = 0.041). **Females:** The DG Fos expression was affected by stress exposure only in the isolated females ($F_{1,12}$= 6,87, p = 0.022). Housing conditions on the other hand, had no effect on Fos-ir in the DG of females in contrast to males. **Gender differences:** Fos-ir was higher in indiv. housed control males than in their female counterparts ($F_{1,13}$ = 10.10, p =0.007). Also the socially housed control ($F_{1,19}$ = 21,38, p< 0.001) and stressed males ($F_{1,19}$ = 8,70, p = 0.008)

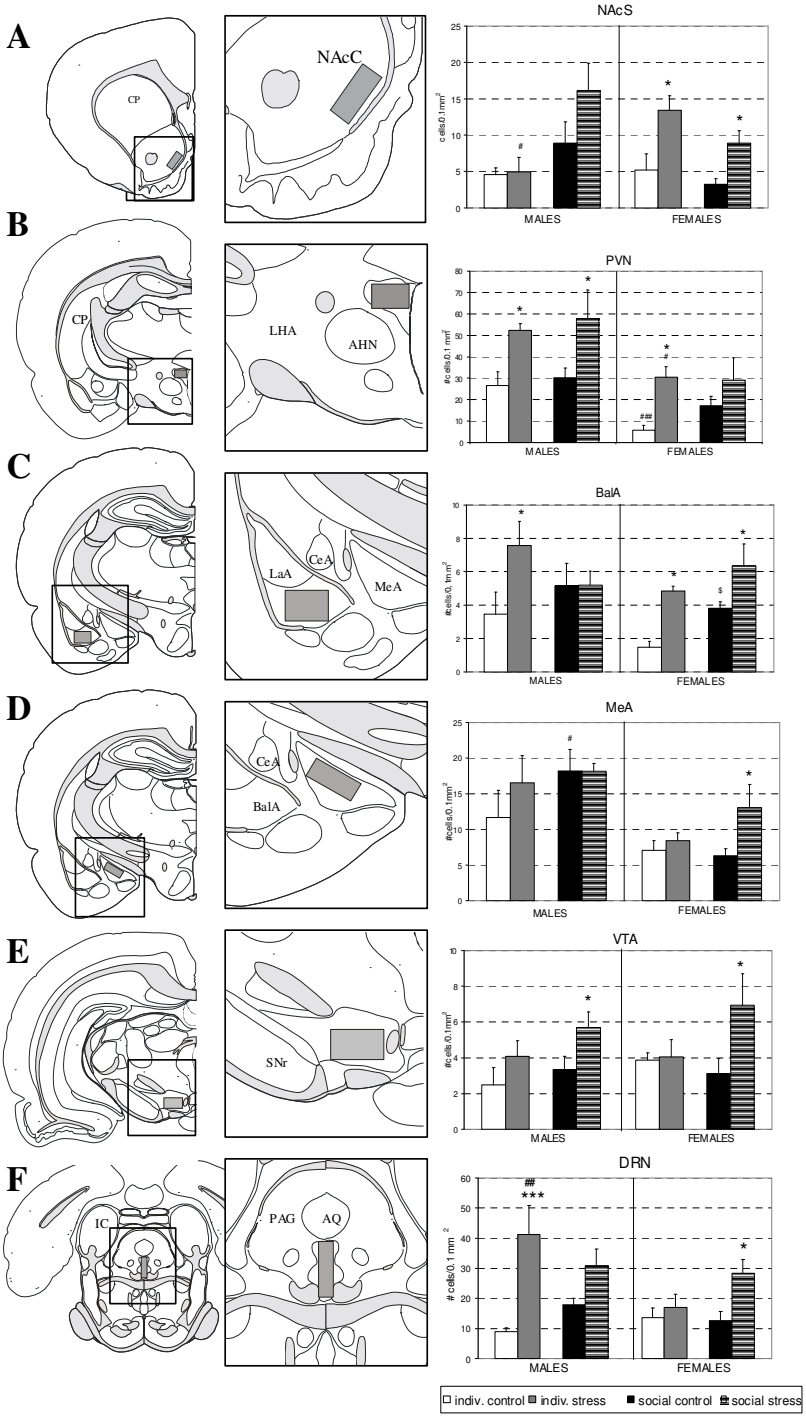
showed more Fos-ir cells in the DG than females in the corresponding groups.

Amygdala: The amygdala, an area involved in emotional processing, showed a significant treatment ($F_{4,27}$ = 2.82, p = 0.045) and gender ($F_{4,27}$ = 3.90, p = 0.013) effect. There was an interaction between gender and housing ($F_{4,27}$ = 3.42, p = 0.022) but only a trend for the interaction between treatment and housing ($F_{4,27}$ = 2.40, p = 0.075). **Males:** Only the LaA ($F_{1,12}$= 5.97, p = 0.031) and BLA ($F_{1,12}$= 8,16, p = 0.014) (Fig. 2C) of isolated males showed a stress-induced increase in the Fos-ir. Individually nor socially housed males showed stress effects in the medial and central nucleus of the amygdala. Social housing during chronic stress exposure led to decreased CeA activity in males ($F_{1,15}$ = 6.15, p =0.025). **Females:** Following stress, isolated females presented an increased activity in the BLA only ($F_{1,15}$ = 8.21, p = 0.012), whereas socially housed females showed increased Fos-ir in the BLA ($F_{1,15}$ = 6.28, p = 0.024) and the MeA ($F_{1,15}$ = 6.82, p = 0.020)(Fig.3D). A housing effect was

observed in the BLA of control females, social housing increasing the Fos-ir ($F_{1,15}$= 4.738, p = 0.046) (Fig. 2C, D). **Gender differences:** Both the isolated control ($F_{1,12}$= 5.80, p =0. 033) and stressed males ($F_{1,15}$= 8,20, p =0.012) showed more Fos-ir in the CeA than their female counterparts. In the LaA a significant gender difference was found for the isolated stressed rats, males showing more Fos-ir than females ($F_{1,12}$= 5.71, p = 0.034). The Fos expression of socially housed control males in the MeA ($F_{1,18}$ = 8.58, p = 0.009)(Fig. 2D) and LaA ($F_{1,15}$ = 10.13, p = 0.006) was higher than in the corresponding female group.

Nucleus Accumbens: The NAc is known for it function in reward motivated behavior and receives dopaminergic input from the VTA. Fos expression in the NAc showed a significant treatment ($F_{2,27}$ = 4.54, p = 0.020), gender ($F_{2,27}$ = 4.13, p = 0.027), housing ($F_{2,27}$ = 3.87, p = 0.033) and gender and housing interaction effect ($F_{2,27}$= 3.54, p = 0.043). The accumbens is composed of two parts, the core and shell areas, each with different connections and functions. In the core region of the accumbens treatment ($F_{1,32}$= 6.18, p= 0.018), gender ($F_{1,32}$= 8.30, p= 0.007) and housing ($F_{1,32}$= 7.30, p = 0.011) had a significant effect on Fos-ir. The shell region showed a treatment ($F_{1,28}$ = 6.75, p = 0.015) and gender/housing interaction

Figure 2. Atlas images of the regions studied. Grey squares represent the quantified regions. The graphs express the mean number of Fos-positive cells per 0.1 mm^2 (±SEM) in the paraventricular nucleus of the hypothalamus (PVN), dorsal raphe nucleus (DRN), shell region of the nucleus accumbens (NacS), ventral tegmental area (VTA), medial (MeA) and basolateral (BLA) nucleus of the amygdala (ac: anterior commissure, AHN: anterior hypothalamic nucleus, AQ: aqueduct, cc: corpus callosum, CP: caudate putamen, IC: inferior colliculus, LHA: lateral hypothalamic area, ml: medial lemniscus, MM: medial mammillary body, opt: optic tract, PAG: periaqueductal gray, RN: red nucleus, SNr: substantia nigra, 3V: third ventricle). Significant effects of chronic stress between rats of the same gender and housing conditions: *, p≤0.05; ***, p≤0.001, significant gender differences within the same treatment and housing groups : $^{\#}$, p≤0.05; $^{\#\#}$, p≤0.01, and significant differences between individually and socially housed rats of the same gender and treatment group: $^{\$}$, p≤0.05.


A
CP
NAcC
NAcS
cells/0.1mm²
MALES
FEMALES
B
CP
LHA
AHN
PVN
MALES
FEMALES
C
LaA
CeA
MeA
BalA
MALES
FEMALES
D
CeA
BalA
MeA
MALES
FEMALES
E
SNr
VTA
MALES
FEMALES
F
IC
PAG
AQ
DRN
MALES
FEMALES
indiv. control
indiv. stress
social control
social stress

effect ($F_{1,28}$= 7.19, p = 0.012). The latter suggests that housing conditions had a different impact on NAcS activity in males and females. **Males:** The NAcC Fos-ir was increased after stress in the isolated males ($F_{1,12}$= 10.13, p = 0.008), but basal expression levels were higher in the socially housed males ($F_{1,16}$ = 6.92, p = 0.018). In contrast ,in the NAcS a non-significant effect of social housing was observed in stressed males; socially housed males showing more Fos-positive cells than isolated males ($F_{1,15}$ = 4.25, p = 0.057) (Fig. 2A). **Females:** Even though a stress effect was observed in the NAcC, pairwise comparisons showed no significant stress effects, albeit that the socially housed females displayed a slightly increased Fos-ir in the NAcC ($F_{1,16}$ = 3.59, p = 0.076). In the NAcS a stress induced increased Fos-ir was found in both the isolated and socially housed females (resp. $F_{1,13}$ = 7.79, p = 0.008; $F_{1,13}$ = 6.82, p = 0.022)(Fig. 2A, 3A). **Gender differences:** The NAcC of the socially housed control males showed a higher Fos-ir than the corresponding female group ($F_{1,20}$ = 6.64, p = 0.018). A gender difference in the NAcS activity was observed between isolated stressed rats ($F_{1,28}$ = 4.21, p = 0.05) (Fig. 2A).

Ventral tegmental area (VTA): The VTA is the origin of the mesolimbic dopaminergic system which is thought to be part of the central reward system. Fos expression in the VTA showed a significant treatment effect ($F_{1,38}$ = 6.43, p= 0.016) (Fig. 2E, 3E). **Males/females:** Stress increased the Fos expression in the VTA of socially housed males ($F_{1,16}$ = 4.97, p= 0.042) and females ($F_{1,15}$ = 5.48, p = 0.033). **Gender differences:** No significant differences in the Fos expression between male and females were found in the VTA.

Dorsal raphe nucleus (DRN): The DRN is the origin of the serotonergic projections to the forebrain. Treatment had a significant effect on Fos-ir in the DRN ($F_{1,27}$ = 19.75, p≤ 0.001) and there was a significant treatment/gender/housing interaction effect ($F_{1,27}$ = 4.81, p = 0.037), indicating that housing conditions and gender influence the way the DRN reacts to stress (Fig. 2F, 3F). **Males/females:** Stress exposure led to increased Fos-ir in the DRN of individually housed males ($F_{1,14}$ = 13.59, p=0.002), but only showed a trend in socially housed males ($F_{1,16}$ = 4,09, p = 0.060). In females the opposite effect of chronic stress exposure was found, namely an increased Fos-ir in socially housed ($F_{1,13}$ = 9.71, p = 0.008) but lack of response with isolation. **Gender difference:** The individually housed stressed male rats expressed more Fos-ir in the DRN than their counterpart females ($F_{1,11}$ = 7,94, p= 0.017).

Discussion

Chronic stress and housing conditions had differential effects on Fos-ir in male and female rats. In general, in males social housing increased basal Fos-ir in several brain areas, and therefore only the PVN and VTA demonstrated a clear stress effect.

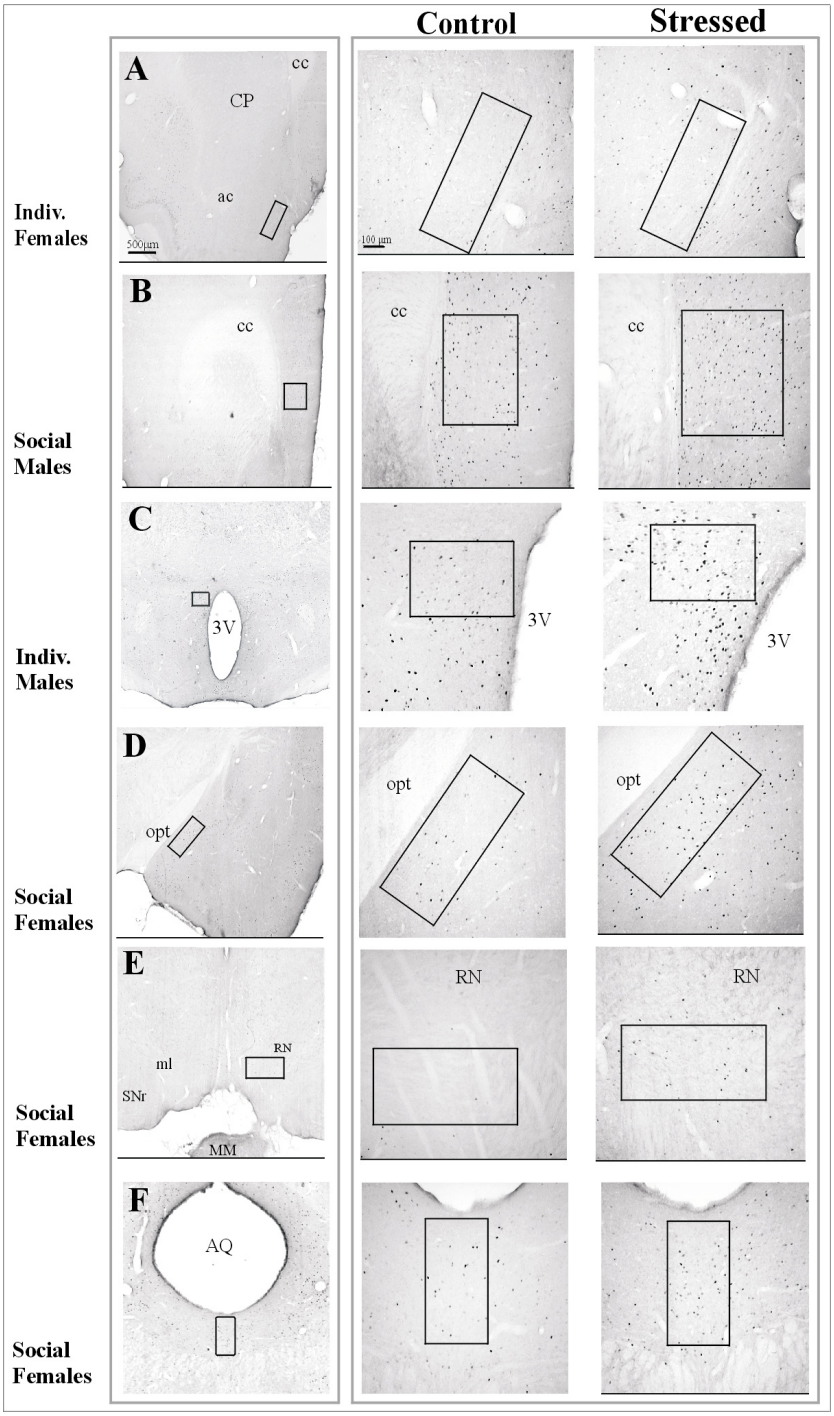


Figure 3. Representative photomicrographs of Fos-ir in NacS (A), IL (B), PVN (C), MeA (D), VTA (E) and DRN (F). See for abbreviations; Figure 2. The first column is at magnification 25x, the second and third at 100x.

Table 2. Number of Fos-positive cells per 0.1mm^2

	male				female			
	individual		social		individual		social	
	control	stress	control	stress	control	stress	control	stress
PVN	26.7 (6.4)	52.4 (3.0)**	30.3 (4.5)	57.7 (13.5)*	5.7 (2.3)$^{\#\#}$	30.4 (5.0)**$^{\#\#}$	17.0 (4.6)	29 (10.5)
PFC								
infralimbic	4.5(1.0)	22.1 (5.7)**	15.1 (2.0)$^{\$\$\$}$	17.3 (2.6)	5.6 (1.4)	11.1 (2.8)$^{\#}$	6.0 (1.0)$^{\#\#\#}$	9.8 (1.1)*
prelimbic	3.7 (0.7)	14.6 (3.6)**	12.5 (1.7)$^{\$\$\$}$	15.3 (2.1)	4.9 (1.0)	11.4 (3.0)	6.6 (0.9)$^{\#\#}$	7.9 (1.6)
cingulate	21.6 (4.4)	90.8 (49.9)*	98.5 (33.8)$^{\$}$	80.0 (24.6)	22.7 (7.6)	49.1 (15.8)$^{\#}$	18.7 (5.8)$^{\#}$	31.2 (7.4)*
dentate gyrus	16.3 (2.4)	19.1(2.2)	40.2 (7.3)$^{\$\$}$	34.9 (2.9)$^{\$}$	8.0 (1.3)$^{\#}$	14.47 (0.5)*	11.18 (2.8)$^{\#\#\#}$	15.48 (2.8)$^{\#\#}$
Amygdala								
central	12.0 (5.5)	21.9 (8.4)	14.0 (3.4)	8.2 (1.5)$^{\$}$	2. (0.8)$^{\#}$	4.5 (1.9)$^{\#}$	6.4 (2.4)	6.9 (2.2)
medial	11.6 (3.8)	16.5 (3.8)	18.2 (3.0)	18.2 (1.1)	7.0 (1.4)	8.4 (1.1)	6.3 (1.0)$^{\#}$	13.1 (2.9)*
lateral	3.6 (1.3)	7.9 (2.0)*	6.1 (0.6)	5.4 (0.3)	2.3 (0.4)	3.7 (0.5)$^{\#}$	2.6 (0.8)$^{\#}$	5.6 (2.9)
basolateral	3.5 (1.3)	7.6 (1.5)*	5.2 (1.3)	5.2 (0.8)	1.5 (0.4)	4.9 (0.3)*	3.8 (0.4)$^{\$}$	6.4 (1.3)*
Accumbens								
core	3.76 (1.4)	11.0 (2.4)**	12.8 (3.0)$^{\$\$}$	13.7 (2.3)	3.2 (0.9)	6.5 (1.3)	5.0 (0.8)$^{\#}$	9.2 (2.5)
shell	4.6 (0.9)	4.9 (2.0)$^{\#}$	8.9 (2.9)	16.1 (3.7)	5.2 (2.2)	13.4 (1.9)*	3.2 (0.8)	8.9 (1.7)*
VTA	3.3 (1.4)	4.1 (0.9)	3.3 (0.7)	5.7 (0.9)*	3.9 (0.4)	5.5 (1.7)	3.1 (0.8)	7.8 (2.0)*
dorsal raphe	8.9 (1.3)	41.2 (9.8)***	17.9 (2.2)	30.9 (5.6)	13.7 (3.2)	17.0 (4.3)$^{\#\#}$	12.6 (3.0)	28.4 (4.5)*

Significant effects of chronic stress between rats of the same gender and housing conditions: *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$, significant gender differences within the same treatment and housing groups : $^{\#}$, $p \leq 0.05$; $^{\#\#}$, $p \leq 0.01$, $^{\#\#\#}$, $p \leq 0.001$ and significant differences between individually and socially housed rats of the same gender and treatment group: $^{\$}$, $p \leq 0.05$; $^{\$\$}$, $p \leq 0.01$; $^{\$\$\$}$. $p \leq 0.001$

In females social housing only increased basal Fos expression in the basolateral nucleus of the amygdala, and thus stress effects on brain activity could be observed in more regions. Chronic stress reduced the growth rate in male but not in female rats, which corroborates other studies.[3,19] Housing conditions did not affect weight gain in males but significantly reduced the growth of control females, a similar non-significant effect was found in stressed females. We attribute this reduced female growth rate not to an increased stress level but to the higher locomotor activity level in the home cage of socially housed females that could be a.o. the result of the heightened activity of oestrous females. Stress induced adrenal hypertrophy was found only in isolated females which also indicates that social housing can lower stress perception in females. Opposite effects of isolation and social housing on adrenal hypertrophy were found in males, and occurred in the socially housed males. This implies that for males social housing by itself is stressful. That chronic stress induced adrenal hypertrophy corresponds with some[2,32] but not all previous work.[19,33] Hypertrophy of the adrenal glands has also been found in depressed patients,[56,61] indicating that adrenal size provides a good measure of the stress perception over periods of time.

Gender specific effects of housing conditions as reported here have also been found in other studies. Basal corticosterone levels were found to be higher in isolated than in socially housed females, while the opposite effect was seen in males.[5] This is corroborated by adrenal hypertrophy in the present study. However a positive influence of social housing on behavioural and physiological stress effects has been

reported also in male rats.[62,76] Possible development of a hierarchy, and subsequent differences in social rank, did probably not occur in the group-housed males, since no fighting or wounds were observed. This is supported by data from the lab of R.R. Sakai (Department of Psychiatry, University of Cincinnati, personal communication), who found no sign of a hierarchy in all-male colonies. Although occurrence of some aggression cannot be excluded and could have provided an additional stress factor for unrelated socially housed males in the current experiment.

We did not specifically investigate effects of the estrus cycle on the stress response.[9] Induction of Fos mRNA by acute restraint stress is differentially affected by stage of the oestrous cycle,[25] but we hypothesised that since females were exposed to the stressor during all stages of the cycle this would overrule sex hormone related stress sensitivity differences, although it might account for some of the variability in Fos expression found in the females. Concordant with previous findings, a synchronisation of the estrus cycle among females was not observed.[63] Females in every group were normal cycling females, it is therefore unlikely that the estrus cycle can explain the differences between the groups. Although females are reported to have a higher stress sensitivity,[77] this is not necessarily reflected by higher Fos-reactivity in the brain.[25]

Another factor explaining the high variability in Fos-ir, as observed in some brain regions, could be a consequence of the study design. We applied the stressor during the dark period, when rats are active. Differences in behavioural activities, like e.g. grooming and social interactions, could have influenced Fos-ir, or the ability of brain regions to show an additional response to exposure to the control or the stress box. Studies investigating stress-related changes of Fos-ir mainly applied the stimulus during the light/resting period, when brain activity is reduced[12] and most likely more homogeneous between rats. More homogeneous and reduced baseline/control levels may lead to a more distinct and less variable stress-related Fos response. In addition, the response to a stressor could be more 'intense' during the resting period because then the nervous system is not primed for stress coping.

Stress induced Fos-ir in isolated males was comparable to patterns shown by Li and Sawchenko after 7 days of footshocks.[12] Apparently 7 days of footshock or the expectancy of a footshock after 21 days induces similar patterns of Fos induction in isolated males. Social housing of males increased Fos-ir in several brain areas, like the PFC, DG and NAcC. Since in socially housed males the PVN activity was not increased we consider it highly unlikely that Fos-ir in the afore mentioned regions is caused by exposure to the control box. Social behaviour occurring among cage mates in the 90 minutes between return to the home cage and sacrifice may have generated the increase basal Fos expression in social control males, since the Fos expression is maximal between 1 and 3 hrs. after a stimulation.[44] This reintroduction

in the home cage may result in a stress-like limbic activation pattern in socially housed control males, which may explain why no beneficial effects of social housing were found in males.

After three weeks exposure to the stress box Fos expression was increased in the PVN[71] and the DRN in most groups, indicating that habituation to the stress protocol did not occur in our model, in contrast to studies that have used chronic restrain stress.[13,66] Inescapable stress, but not escapable stress activates DRN 5HT neurons,[31,48] and rats undergoing 'psychological' stress showed increased 5HT release in the DRN.[26] The increased Fos expression in the DRN in the present study suggests that serotonergic raphe neurons are activated in response to the 'psychological' component of the exposure to the stress box. While Fos-ir in the PVN of isolated females was increased after stress exposure, the DRN of these animals was non-responsive. This may be related to a lack of activation of the serotonergic system during stress whereas social housing prevented this dysfunction like a 'natural antidepressant', maintaining the reactivity of the serotonergic system during chronic stress.

Oxytocin could be a possible mediator of the positive effects of social housing, since it is involved in social behavior in rodents[10,37] and can reduce the stress response.[58,59,80] Social contact in rats has been shown to increase oxytocin release.[75] In humans social contact is also associated with increases in plasma oxytocin levels[73] and has been found to reduce plasma cortisol levels.[10,21] It has been hypothesised that women, and possibly also female rats, may seek social contact during stress, which could increase oxytocin levels, that could be acting as an endogenous anti-stress system.[68] In the present study the slightly higher basal Fos expression in the PVN of socially housed females compared to isolated females may be related to increased oxytocin release resulting from social behaviour, like allogrooming, after re-exposure to cage mates. D-fenfluramine, which increases levels of serotonin, has been found to elicit Fos-ir in oxytocin cells in the PVN.[38] Hypothetically, the stress-induced DRN activity could have stimulated oxytocin release, which subsequently decreased the stress sensitivity in socially housed females and isolated males.

The amygdala is thought to be involved in processing and the expression of emotional stimuli[14,57] and is activated with the presentation of emotionally negative pictures in humans.[8] Depressed patients show an altered amygdala activity.[18,65] In rodents the BLA and LaA are associated with fear conditioning and memory retrieval[45,51,52] and the medial amygdala is involved in the regulation of the HPA-axis.[15] Since social housing ameliorated stress coping in females,[78] we expected reduced stress-induced Fos-ir in the amygdala of socially housed females. However in the MeA and BLA Fos-ir was increased suggesting that these amygdala nuclei of socially housed females were more affected by stress. Lesioning of the MeA

has been shown to block the activation of oxytocin cells in the PVN, implying that activation of the MeA can induce oxytocin release.[15] Oxytocin released in the MeA has been shown to be involved in social recognition.[24] It is possible therefor that stress-induced activation of the MeA, as seen in socially housed females, promotes social recognition of cage mates, which reduces the impact of stressful conditions. The BLA and LaA of isolated stressed males showed increased Fos-ir expression. Surprisingly this effect was absent in socially housed males. The latter animals probably were used to a more dynamic environment and therefor less anxious even though they apparently were more affected by chronic stress than isolated males. Both isolated and socially housed females show a stress-induced increase of BLA activity, but socially housed controls have an higher basal Fos expression level. Separation from their cage mates may be perceived as a stressful condition, increasing Fos-ir in the BLA and PVN.

Disturbances in the mesolimbic dopaminergic reward system are thought to underlie the inability to experience pleasure, a core symptom of depression.[18,55,57] Increased mesolimbic DA release appears to be related to active coping with an aversive stimulus, whereas inhibition of mesolimbic DA release occurs with uncontrollable/unavoidable stressors and failure to cope.[6] Also in chronic stress models changes in dopaminergic activity have been observed.[6,17] Acute stressful stimuli increased DA levels specifically in the shell region of the accumbens[39,81] whereas impaired DA reactivity and reduced basal DA levels were found after 3 weeks of stress,[46] which was reversible by antidepressant treatment.[28] In the present study the dopaminergic system of isolated males apparently was hypoactive, since stress-induced increases in Fos-ir in the VTA[54] and accumbens shell were not found. Socially housed males however did show a stress-induced increased Fos expression in the VTA and even though the NAcS did not show a stress-induced activation, NAcS Fos-ir was increased by social housing. If dopaminergic system non-response to adverse events is characteristic for depressive symptomatology, our results would suggest that socially housed males can cope better with chronic stress than isolated males. Alternatively, increased VTA activity in these males could also reflect an attempt to cope, unsuccessfully when looking at behaviour and adrenal size,[78] with the inescapable footshocks, even after 3 weeks. It is temping to speculate that animals who prolong the endeavour to cope will put additional strain on the nervous system compared to animals who show resignation to the stressor in an earlier stage, which appears to be influenced by housing conditions. Female rats showed a different mesoaccumbens response to chronic stress. The VTA of isolated females did not respond to stress in contrast with that of socially housed females, suggesting a hypoactive DA-ergic system in isolated females. However the NAcS, which is innervated by VTA DA-ergic neurons,[74] did show a response to stress in

isolated females. A possible mechanism for the increased activity in the NAcS could be a hypersensitivity to DA, possibly involving increased numbers of DA receptors. There are indications for altered dopamine receptor densities in depression, although results are inconsistent.[20,42] A study by Tremblay and co-workers[70] found indications for a hypersensitive response of the brain reward system to dextroamphetamine, possibly reflecting a hypofunctional state. A similar change might have occurred in isolated females during chronic stress exposure. Socially housed females showed a stress-induced activation of the VTA, likely activating the accumbens shell due to DA release, similar as to what has been found with acute stress.[39,81] This could point towards a normal stress-induced activation of the mesoaccumbens dopaminergic system in socially housed females, in contrast with isolated females.

Summarising, the limbic system shows a gender specific activation pattern in response chronic stress and housing conditions. Female rats seem to benefit from social housing although the possible neurobiological mechanism does not appear to be simply a reduction or prevention of the stress-induced Fos expression as observed in isolated females. Also in males, where social housing seems to deteriorate stress coping, Fos-ir expression was not just increased compared to individually housed stressed males. Since almost all neuropharmacological studies in rats have been performed in male rats, interpreting the found responses in females is rather difficult. More research is needed to clarify the neurobiological mechanisms of stress coping in females, since they appear to be quite different from males.

References

1. **Beck CH, Fibiger HC**. Chronic desipramine alters stress-induced behaviors and regional expression of the immediate early gene, c-fos. *Pharmacol Biochem Behav* 1995;51:331-338
2. **Blanchard RJ, Nikulina JN, Sakai RR, McKittrick C, McEwen B, Blanchard DC**. Behavioral and endocrine change following chronic predatory stress. *Physiol Behav* 1998;63:561-569
3. **Bowman RE, Zrull MC, Luine VN**. Chronic restraint stress enhances radial arm maze performance in female rats. *Brain Res* 2001;904:279-289
4. **Brody AL, Saxena S, Stoessel P, et al.** Regional brain metabolic changes in patients with major depression treated with either paroxetine or interpersonal therapy: preliminary findings. *Arch Gen Psychiatry* 2001;58:631-640
5. **Brown KJ, Grunberg NE**. Effects of housing on male and female rats: crowding stresses male but calm females. *Physiol Behav* 1995;58:1085-1089
6. **Cabib S, Puglisi-Allegra S**. Stress, depression and the mesolimbic dopamine system. *Psychopharmacology (Berl)* 1996;128:331-342
7. **Canli T, Desmond JE, Zhao Z, Gabrieli JD**. Sex differences in the neural basis of emotional memories. *Proc Natl Acad Sci U S A* 2002;99:10789-10794
8. **Canli T, Sivers H, Whitfield SL, Gotlib IH, Gabrieli JD**. Amygdala response to happy faces as a function of extraversion. *Science* 2002;296:2191
9. **Carey MP, Deterd CH, de Koning J, Helmerhorst F, de Kloet ER**. The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *J Endocrinol* 1995;144: 311-321
10. **Carter CS**. Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 1998;23:779-818
11. **Cheeta S, Ruigt G, van Proosdij J, Willner P**. Changes in sleep architecture following chronic

mild stress. *Biol Psychiatry* 1997;41:419-427
12. **Cirelli C, Tononi G**. On the functional significance of c-fos induction during the sleep-waking cycle. *Sleep* 2000;23:453-469
13. **Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ**. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* 1995;64:477-505
14. **Davidson RJ**. Anxiety and affective style: role of prefrontal cortex and amygdala. *Biol Psychiatry* 2002;51:68-80
15. **Dayas CV, Buller KM, Day TA**. Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. *Eur J Neurosci* 1999;11:2312-2322
16. **de Jonghe F, Kool S, van Aalst G, Dekker J, Peen J**. Combining psychotherapy and antidepressants in the treatment of depression. *J Affect Disord* 2001;64:217-229
17. **Di Chiara G, Loddo P, Tanda G**. Reciprocal changes in prefrontal and limbic dopamine responsiveness to aversive and rewarding stimuli after chronic mild stress: implications for the psychobiology of depression. *Biol Psychiatry* 1999;46:1624-1633
18. **Drevets WC**. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. *Curr Opin Neurobiol* 2001;11:240-249
19. **Duncko R, Kiss A, Skultetyova I, Rusnak M, Jezova D**. Corticotropin-releasing hormone mRNA levels in response to chronic mild stress rise in male but not in female rats while tyrosine hydroxylase mRNA levels decrease in both sexes. *Psychoneuroendocrinology* 2001;26:77-89
20. **Ebert D, Feistel H, Loew T, Pirner A**. Dopamine and depression--striatal dopamine D2 receptor SPECT before and after antidepressant therapy. *Psychopharmacology (Berl)* 1996;126:91-94
21. **Evans JJ**. Oxytocin in the human--regulation of derivations and destinations. *Eur J Endocrinol* 1997;137:559-571
22. **Ezquiaga E, Garcia A, Pallares T, Bravo MF**. Psychosocial predictors of outcome in major depression: a prospective 12-month study. *J Affect Disord* 1999;52:209-216
23. **Fallon JH, Loughlin SE**. *Substantia Nigra.* In:Paxinos G, ed. The Rat Nervous System. Academic Press, 1995:215-237
24. **Ferguson JN, Aldag JM, Insel TR, Young LJ**. Oxytocin in the medial amygdala is essential for social recognition in the mouse. *J Neurosci* 2001;21:8278-8285
25. **Figueiredo HF, Dolgas CM, Herman JP**. Stress activation of cortex and hippocampus is modulated by sex and stage of estrus. *Endocrinology* 2002;143:2534-2540
26. **Funada M, Hara C**. Differential effects of psychological stress on activation of the 5-hydroxytryptamine- and dopamine-containing neurons in the brain of freely moving rats. *Brain Res* 2001;901:247-251
27. **Galea LA, McEwen BS, Tanapat P, Deak T, Spencer RL, Dhabhar FS**. Sex differences in dendritic atrophy of CA3 pyramidal neurons in response to chronic restraint stress. *Neuroscience* 1997;81:689-697
28. **Gambarana C, Masi F, Tagliamonte A, Scheggi S, Ghiglieri O, De Montis MG**. A chronic stress that impairs reactivity in rats also decreases dopaminergic transmission in the nucleus accumbens: a microdialysis study. *J Neurochem* 1999;72:2039-2046
29. **George MS, Ketter TA, Parekh PI, Herscovitch P, Post RM**. Gender differences in regional cerebral blood flow during transient self-induced sadness or happiness. *Biol Psychiatry* 1996;40: 859-871
30. **Glynn LM, Christenfeld N, Gerin W**. Gender, social support, and cardiovascular responses to stress. *Psychosom Med* 1999;61:234-242
31. **Grahn RE, Will MJ, Hammack SE, et al.** Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor. *Brain Res* 1999;826:35-43
32. **Haller J, Fuchs E, Halasz J, Makara GB**. Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res Bull* 1999;50:33-39
33. **Harro J, Haidkind R, Harro M, et al.** Chronic mild unpredictable stress after noradrenergic denervation: attenuation of behavioural and biochemical effects of DSP-4 treatment. *Eur Neuropsychopharmacol* 1999;10:5-16
34. **Hirschfeld RM, Dunner DL, Keitner G, et al.** Does psychosocial functioning improve independent of depressive symptoms? A comparison of nefazodone, psychotherapy, and their combination. *Biol Psychiatry* 2002;51:123-133
35. **Hogan BE, Linden W, Najarian B**. Social support interventions Do they work? *Clinical Psychology Review* 2002;22:381-440
36. **Holscher C**. Stress impairs performance in spatial water maze learning tasks. *Behav Brain Res* 1999;100:225-235
37. **Insel TR, Winslow JT**. Serotonin and neuropeptides in affiliative behaviors. *Biol Psychiatry* 1998;44:207-219
38. **Javed A, Kamradt MC, Van de Kar LD, Gray TS**. D-Fenfluramine induces serotonin-mediated Fos expression in corticotropin-releasing factor and oxytocin neurons of the hypothalamus, and serotonin-independent Fos expression in enkephalin and neurotensin neurons of the amygdala. *Neuroscience* 1999;90:851-858
39. **Kalivas PW, Duffy P**. Selective activation of dopamine transmission in the shell of the nucleus

accumbens by stress. *Brain Res* 1995;675:325-328
40. **Kessler RC, McGonagle KA, Swartz M, Blazer DG, Nelson CB**. Sex and depression in the National Comorbidity Survey. I: Lifetime prevalence, chronicity and recurrence. *J Affect Disord* 1993;29:85-96
41. **Kirschbaum C, Klauer T, Filipp SH, Hellhammer DH**. Sex-specific effects of social support on cortisol and subjective responses to acute psychological stress. *Psychosom Med* 1995;57:23-31
42. **Klimke A, Larisch R, Janz A, Vosberg H, Muller-Gartner HW, Gaebel W**. Dopamine D2 receptor binding before and after treatment of major depression measured by [123I]IBZM SPECT. *Psychiatry Res* 1999;90:91-101
43. **Kornstein SG, Schatzberg AF, Thase ME, et al.** Gender differences in treatment response to sertraline versus imipramine in chronic depression. *Am J Psychiatry* 2000;157:1445-1452
44. **Kovacs KJ**. c-Fos as a transcription factor: a stressful (re)view from a functional map. *Neurochem Int* 1998;33:287-297
45. **LeDoux JE**. Emotion circuits in the brain. *Annu Rev Neurosci* 2000;23:155-184
46. **Mangiavacchi S, Masi F, Scheggi S, Leggio B, De Montis MG, Gambarana C**. Long-term behavioral and neurochemical effects of chronic stress exposure in rats. *J Neurochem* 2001;79: 1113-1121
47. **Martenyi F, Dossenbach M, Mraz K, Metcalfe S**. Gender differences in the efficacy of fluoxetine and maprotiline in depressed patients: a double-blind trial of antidepressants with serotonergic or norepinephrinergic reuptake inhibition profile. *Eur Neuropsychopharmacol* 2001;11:227-232
48. **Maswood S, Barter JE, Watkins LR, Maier SF**. Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat. *Brain Res* 1998;783: 115-120
49. **Matthews K, Forbes N, Reid IC**. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav* 1995;57:241-248
50. **McEwen BS**. *The effects of stress on structural and functional plasticity in the hippocampus.* In: Charney DS, Nestler E J, Bunney B S, eds. Neurobiology of Mental Illness. New York: Oxford university press, 1999:475-493
51. **McGaugh J**. Memory consolidation and the amygdala: a systems perspective. *Trends Neurosci* 2002;25:456
52. **McGaugh JL, Roozendaal B**. Role of adrenal stress hormones in forming lasting memories in the brain. *Curr Opin Neurobiol* 2002;12:205-210
53. **Morinobu S, Nibuya M, Duman RS**. Chronic antidepressant treatment down-regulates the induction of c-fos mRNA in response to acute stress in rat frontal cortex. *Neuropsychopharmacology* 1995;12:221-228
54. **Morrow BA, Elsworth JD, Lee EJ, Roth RH**. Divergent effects of putative anxiolytics on stress-induced fos expression in the mesoprefrontal system of the rat. *Synapse* 2000;36:143-154
55. **Naranjo CA, Tremblay LK, Busto UE**. The role of the brain reward system in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2001;25:781-823
56. **Nemeroff CB, Krishnan KR, Reed D, Leder R, Beam C, Dunnick NR**. Adrenal gland enlargement in major depression. A computed tomographic study. *Arch Gen Psychiatry* 1992;49:384-387
57. **Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM**. Neurobiology of depression. *Neuron* 2002;34:13-25
58. **Neumann ID, Kromer SA, Toschi N, Ebner K**. Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. *Regul Pept* 2000;96:31-38
59. **Petersson M, Hulting AL, Uvnas-Moberg K**. Oxytocin causes a sustained decrease in plasma levels of corticosterone in rats. *Neurosci Lett* 1999;264:41-44
60. **Quintana SM, Maxwell SE**. A Monte Carlo comparison of seven e-adjustments procedures in repeated measures designs with small sample sizes. *Journal of Educational Statistics* 1994;19:57-71
61. **Rubin RT, Phillips JJ, McCracken JT, Sadow TF**. Adrenal gland volume in major depression: relationship to basal and stimulated pituitary-adrenal cortical axis function. *Biol Psychiatry* 1996;40:89-97
62. **Ruis MA, te Brake JH, Buwalda B, et al.** Housing familiar male wildtype rats together reduces the long-term adverse behavioural and physiological effects of social defeat. *Psychoneuroendocrinology* 1999;24:285-300
63. **Schank JC**. Do Norway rats (Rattus norvegicus) synchronize their estrous cycles? *Physiol Behav* 2001;72:129-139
64. **Senba E, Ueyama T**. Stress-induced expression of immediate early genes in the brain and peripheral organs of the rat. *Neurosci Res* 1997;29:183-207
65. **Siegle GJ, Steinhauer SR, Thase ME, Stenger VA, Carter CS**. Can't shake that feeling: event-related fMRI assessment of sustained amygdala activity in response to emotional information in depressed individuals. *Biol Psychiatry* 2002;51:693-707
66. **Stamp JA, Herbert J**. Multiple immediate-early gene expression during physiological and endocrine adaptation to repeated stress. *Neuroscience* 1999;94:1313-1322
67. **Swanson LW**. *Brain Maps: Structure of the rat brain.* Amsterdam: Elsevier, 1992:

68. **Taylor SE, Klein LC, Lewis BP, Gruenewald TL, Gurung RA, Updegraff JA**. Biobehavioral responses to stress in females: tend-and-befriend, not fight-or-flight. *Psychol Rev* 2000;107:411-429
69. **Ter Horst GJ, Meijler WJ, Korf J, Kemper RH**. Trigeminal nociception-induced cerebral Fos expression in the conscious rat. *Cephalalgia* 2001;21:963-975
70. **Tremblay LK, Naranjo CA, Cardenas L, Herrmann N, Busto UE**. Probing brain reward system function in major depressive disorder: altered response to dextroamphetamine. *Arch Gen Psychiatry* 2002;59:409-416
71. **Trentani A, Kuipers SD, Ter Horst GJ, Den Boer JA**. Selective chronic stress-induced in vivo ERK1/2 hyperphosphorylation in medial prefrontocortical dendrites: implications for stress-related cortical pathology? *Eur J Neurosci* 2002;15:1681-1691
72. **Troisi A**. Gender differences in vulnerability to social stress. A Darwinian perspective. *Physiol Behav* 2001;73:443-449
73. **Turner RA, Altemus M, Enos T, Cooper B, McGuinness T**. Preliminary research on plasma oxytocin in normal cycling women: investigating emotion and interpersonal distress. *Psychiatry* 1999;62:97-113
74. **Tzschentke TM**. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. *Prog Neurobiol* 2001;63:241-320
75. **Uvnas-Moberg K**. Physiological and endocrine effects of social contact. *Ann N Y Acad Sci* 1997;807:146-163
76. **Von Frijtag JC, Reijmers LG, Van der Harst JE, Leus IE, Van den BR, Spruijt BM**. Defeat followed by individual housing results in long-term impaired re. *Behav Brain Res* 2000;117:137-146
77. **Weinstock M, Razin M, Schorer-Apelbaum D, Men D, McCarty R**. Gender differences in sympathoadrenal activity in rats at rest and in response to footshock stress. *Int J Dev Neurosci* 1998;16:289-295
78. **Westenbroek C, Ter Horst GJ, Roos MH, Kuipers SD, Trentani A, Den Boer JA**. Gender-specific effects of social housing in rats after chronic mild stress exposure. *Prog Neuropsychopharmacol Biol Psychiatry* 2003;27:21-30
79. **Willner P**. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* 1997;134:319-329
80. **Windle RJ, Shanks N, Lightman SL, Ingram CD**. Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. *Endocrinology* 1997;138:2829-2834
81. **Wu YL, Yoshida M, Emoto H, Tanaka M**. Psychological stress selectively increases extracellular dopamine in the 'shell', but not in the 'core' of the rat nucleus accumbens: a novel dual-needle probe simultaneous microdialysis study. *Neurosci Lett* 1999;275:69-72